

Functional traits of medicinal plant species under different ecological conditions

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Abstract

Traits and attributes of plants in plant communities are the outcome of adaptation of species to the environment and acclimation to environmental conditions changing in space and time. This is especially true for medicinal plants, which can affect the surrounding plants with their properties. Identification of plant functional groups in plant community can provide a better understanding and functional comparison of several communities than the classical approach based on taxonomy. To compare the effect of different ecological conditions (one control and three experimental sites in Bratislava, Slovakia) on the plasticity of medicinal plant species, and on the plant functional groups based on the selected leaf functional traits – SLA (specific leaf area) and LDMC (leaf dry matter content). In assessment were tested thirteen medicinal plant species – *Aegopodium podagraria* L., *Fragaria vesca* L., *Galium odoratum* (L.) Scop., *Geum urbanum* L., *Glechoma hederacea* L., *Hedera helix* L., *Hypericum perforatum* L., *Impatiens glandulifera* Royle, *Plantago lanceolata* L., *Prunella vulgaris* L., *Solidago gigantea* Aiton, *Tussilago farfara* L. and *Urtica dioica* L. For the formation of plant functional groups according to different leaf characteristics, the metric multidimensional scaling (MDS) ordination diagram was applied. Photosynthetically active radiation (PAR) had a more significant effect on studied leaf traits than content of soil organic matter (SOC) (their coefficients of determination had values 0.55 and 0.11). Studied species were also characterized according to plant strategies. *Urtica dioica* and *Geum urbanum* possessed the greatest plasticity of all studied species.

Introduction

There is growing recognition that classifying terrestrial plant species based on their function rather than their taxonomic identity is a promising way forward to tackling important ecological questions at the scale of ecosystems, landscapes or biomes (e.g., Cornelissen et al. 2003). These questions include vegetation responses to and vegetation effects on environmental change, such as climatic change, atmospheric chemistry or land use. According to Hulshof and Swenson (2010), patterns of species coexistence and species diversity in plant communities remain an important research area despite over a century of intensive study. To provide mechanistic insight into the rules governing plant species coexistence and diversity, plant community ecologists are increasingly quantifying functional trait values for species growing in a range of communities.

Plant functional traits are the features (morphological, physiological, phenological) that represent ecological strategies and determine how plants respond to environmental factors (Amaral et al. 2021), affect other trophic levels and influence ecosystem properties (Pérez-Harguindeguy et al. 2013). Variation in plant functional traits results from evolutionary and environmental drivers that operate at multiple scales, making it challenging to distinguish among them (Reich et al. 2003). Thus, the functional-trait approach to communities focuses on the distribution of traits and their variation in communities along environmental gradients (Bédécarrats and Isselin-Nondedeu 2012, Pérez-Ramos et al. 2019, Liu et al. 2021). Since morphological traits, such as leaf area, SLA and LDMC, reflect the leaf economic spectrum and plant adaptation (Wright et al. 2004) as well as they are used to classified ecological strategies

according to CSR theory (Grime 1977) SLA and LDMC were used for our study. Moreover, according to Lambers and Poorter (1992), a high leaf SLA represents a resource-acquisitive plant strategy, while a high leaf dry matter content (LDMC) represents a resource-conservative strategy. Hodgson et al. (2011) stressed that SLA is preferred plant trait for assessing soil fertility, whereby SLA is a function of LDMC and leaf thickness. Although leaf density is strongly related to LDMC, and LDMC and leaf thickness each contribute equally to the expression of SLA, the exact relationships differ between ecological groupings. LDMC predicts leaf nitrogen content and soil fertility but, because leaf thickness primarily varies with irradiance, SLA increases in response to both increased shade and increased fertility (Smart et al. 2017, Dalke et al. 2018). Therefore SLA, which includes both fertility and shade components, may often discriminate better between communities or treatments than LDMC.

Based on these relationships we focused on the photosynthetically active radiation (PAR) and soil organic matter (SOM) from studied ecological factors. The level of irradiance is an important ecological factor on which all photo-autotrophic plants depend. Low light intensities pose stresses on plants because irradiance limits photosynthesis and thus net carbon gain and plant growth (Lambers and Oliveira 2019, Gitelson et al. 2021). Content of the soil organic carbon is an essential characteristic that affects soil fertility and texture (Scharlemann 2014) with its complex and heterogeneous structures although it occupies a minor percentage of the soil weight (Khaled and Fawy 2011).

Phenotypic plasticity can be characterised as a possibility of a single genotype to produce different phenotypes in diverse environments (Schlichting 1986, Schlichting and Smith 2002, Sultan 2000). Recently, intensive study has shown that plants are plastic for a remarkable array of ecologically important traits (Nicotra et al. 2010, Wang et Zhou 2021), ranging from diverse aspects of morphology and physiology to anatomy, developmental and reproductive timing, offspring developmental patterns, and breeding system. The ability of an organism to change its phenotype in response to diverse environments, termed plasticity, is an essential characteristic for sessile plants to acclimate to rapid changes in their environment (Stotz et al. 2021). Plasticity is a quantitative trait that can provide a fitness advantage and mitigate negative effects due to environmental perturbations (Laitinen and Nikoloski 2019, Mackenzie and Kundariya 2019).

Species can be clustered into plant functional groups, i.e. sets of species that share similar characteristics within a community. Plant species of a functional group respond similarly to environmental pressures and ecosystem processes and habitat properties (Mahdavi and Bergmeier 2016). Functional groups serve as a strategy for representing plant communities and their relationships with the biotic and abiotic environment, without the need for data input for each species (Rogers et al. 2019). In community ecology, plant functional groups are widely used to describe trait variation within and across plant communities (Thomas et al. 2019). It has long been a central tenet of ecology that environmental conditions are largely responsible for the combinations of traits that have been selected for in species and for classifying species into assemblages (Craine 2005). The Grime's CSR (Grime 2001) model constitutes one of the most established systems for plant functional types. The primary strategies

(competitive ability, adaptation to stress and adaptation to disturbance) relate to level of disturbance and the productivity at a given site (Schmidtlein et al. 2012).

Medicinal plants synthesize many biologically active chemical compounds (secondary metabolites) (Li et al. 2020), that are important for defence against insects, fungi, diseases, and herbivorous animals. Moreover, in a natural plant community, they markedly affect other species through allelopathy. Wild medicinal plants are thus not only a substantial component of natural plant community, but also responsible for biodiversity and stability of natural ecosystems (Masarovičová et al. 2019).

Based on the importance of medicinal plant species for natural plant community, the aim of our research was: (i) to identify the trait plasticity using two functional traits (specific leaf area (SLA) and leaf dry matter content (LDMC)) for all 13 studied species; (ii) to classify species into functional groups based on their leaf characteristics within a community; (iii) to characterize the species from the perspective of Grime's plant strategies; (iv) to discuss the relationship between PAR or soil organic matter content and leaf characteristics.

Methods

Research site characteristics

Four research sites in the region of the city Bratislava (SW Slovakia) were chosen – the Garden of Medicinal Plants, Faculty of Pharmacy, Comenius University in Bratislava (BG) (GPS coordinates: 48.1419761N; 17.1894686E), a meadow in the Bratislava district Rača (R) (GPS coordinates: 48.2266050N; 17.1430978E), a forest in the Bratislava district Podunajské Biskupice (PB) (GPS coordinates: 48.0799625N; 17.2022244E) and a site located close to the Faculty of Electrical engineering and Information technology STU in Bratislava (ST) (GPS coordinates: 48.1530903N; 17.0723700E). In (BG), all 13 species were sampled. In each of (R), (PB), and (ST), a subset of the species was sampled, whereby these subsets were not overlapping.

The first research site (BG) served as a control site. Approx. 850 medicinal plant species or potentially medicinal plants are grown in the garden, and the plants are used for both research and education. The garden represents a site with regular management (cultivation, fertilization, and irrigation). Thirteen medicinal plant species from this site were used: *Aegopodium podagraria* L., *Fragaria vesca* L., *Galium odoratum* (L.) Scop., *Geum urbanum* L., *Glechoma hederacea* L., *Hedera helix* L., *Hypericum perforatum* L., *Impatiens glandulifera* Royle, *Plantago lanceolata* L., *Prunella vulgaris* L., *Solidago gigantea* Aiton, *Tussilago farfara* L., and *Urtica dioica* L. The studied medicinal species were chosen based on previous experiences and because they occurred in the control site (BG) and in another experimental site at the same time.

The second research site (R), represented a meadow close to a forest. The following experimental species were sampled: *Aegopodium podagraria*, *Impatiens glandulifera*, *Solidago gigantea*, *Tussilago farfara*, *Fragaria vesca*, and *Prunella vulgaris*.

The third experimental site was an alluvial forest (PB). This forest represented a more shaded site than the previous two sites with a higher humus content. Five plant species *Galium odoratum*, *Geum urbanum*, *Hedera helix*, *Glechoma hederacea*, and *Urtica dioica*, were sampled.

The final experimental research site was an anthropogenically influenced biotope (ST). This site was without regular irrigation, with occasional mowing, but had the lowest soil organic matter. Samples were taken of *Hypericum perforatum* and *Plantago lanceolata*.

Fieldwork

The research was conducted in 2018 and 2019. For the measurement of leaf functional traits, we used fully expanded physiologically mature leaves. The leaves were representative and free from herbivore or pathogen damage. If possible, we took 10 samples from each species from each site. Following Cornelissen et al. (2003), we cut the leaf with a petiole as close to a stem as possible. Some authors (e.g. Rodriguez et al. 2015, Rosbakh et al. 2015) consider the petiole and a vein as a part of the leaf. However, other authors (e.g., Garnier et al. 2001, Liu et al. 2017, Kattenborn et al. 2019) measured the SLA of the leaf lamina without the petiole. As we were working on our project, we found that the samples of simple leaves with the petioles showed lower values of SLA and LDMC than samples of simple leaves without petioles. These results show that petioles have a significant influence on the tested functional leaf characteristics. Therefore, we used leaves without the petioles.

On each above-mentioned site, PAR was measured ($\mu\text{mol m}^{-2} \text{s}^{-1}$, MQS Cosine Corrected Mini Quantum Sensor, ICT International, Armidale, NSW, Australia), and soil samples were taken at a 0 – 10 cm depth.

Plant samples processing

The measurement of the functional traits of the plants was in accordance with the procedure described in Cornelissen et al. (2003). The leaf characteristics of the plants that have been determined include: FM - fresh leaf weight [kg], DM - dry weight of the leaf [kg], LA - leaf area [m²].

To determine SLA and LDMC values, the following formulas were applied:

$$\text{SLA} = \text{LA}/\text{DM} [\text{m}^2 \text{kg}^{-1}]$$

$$\text{LDMC} = \text{DM}/\text{FM} [\text{kg kg}^{-1}]$$

Soil sample processing

Soil samples were air dried and passed through a 2-mm sieve prior to analysis. Soil organic carbon (SOC) was determined by potassium dichromate oxidation (Nelson and Sommers 1996). The percentage of soil organic matter (SOM) was calculated from SOC using the conversion factor 1.724 (Nair 1993). Soil texture was determined by the pipette method (Gee and Bauder 2002) and the soil textural class was

found using the USDA textural triangle (USDA 1987). Soil pH was measured in a 1:2.5 soil:0.01 M CaCl₂ suspension.

Data analysis

For both analysed leaf traits (SLA and LDMC), the mean values and bootstrapped 95 % confidence intervals were determined for each plant species at the control and experimental site. To calculate the bootstrap confidence intervals (bootstrap replicates = 1000), the “boot” package was used (Canty and Ripley 2021).

A Gower dissimilarity matrix based on leaf trait values of each species was used in the metric multidimensional scaling (MDS). The MDS was carried out to identify the position of species centroids at each sampling site and to determine the shift of species centroids between control and experimental site, as well as to evaluate leaf trait plasticity for each species (bivariate ellipse - standard deviation + 95 % confidence limits). To the created ordination diagram, PAR and soil organic matter were projected as supplementary variables.

The same Gower dissimilarity was used in the MDS to determine species groups based on the analysed leaf traits. Generalized additive models with a thin plate spline were used to predict and plot the surface of the PAR and soil organic matter gradient on the MDS ordination diagram. In the MDS plot, values of classification trait criteria were obtained from classification and regression tree analyses (CART). The result of the CART was projected to the ordination diagram through the orthogonal projection of each trait classification criterion on the trait vector in the ordination. In the CART model, the relevant number of splits was controlled by the complexity parameter (cp), which imposes a penalty to the tree for having too many splits (i.e. if the next splitting does not significantly improve the overall quality of the previous model). The cp value was set to 0.01. The MDS and CART were performed in the “Vegan 2.5-7” package (Oksanen et al. 2020) and “rpart 4.1-15” package (Therneau and Atkinson 2019), respectively. All used packages were run under the R 3.6.3 (R Core Team 2020) software environment.

Results

SLA and LDMC

For the studied species, mean values of SLA ranged from 10.3 m² kg⁻¹ to 66.3 m² kg⁻¹ (Fig. 1a). The highest within-site variability in the SLA values were mainly found in the population of the plant species which had a mean value of the SLA higher than 35 m² kg⁻¹ (e.g., *Impatiens glandulifera* (R), *Glechoma hederacea* (BG, PB)). The mean values of LDMC were between 0.12 kg kg⁻¹ and 0.38 kg kg⁻¹. Similar to SLA, the species within-site variability in the LDMC values increased with the increase of the mean value of this parameter. Significantly highest within-site variability was especially found in *Urtica dioica* in the botanic garden (Fig. 1b). According to the overlap of the bootstrapped 95% confidence interval of the ordered mean values, six groups were identified for SLA, and four groups for LDMC.

Leaf Trait Plasticity And Classification Of Functional Groups

For the evaluation of species plasticity in analysed leaf traits, MDS was applied (Fig. 2).

Figure 2 Results of the MDS showing the species plasticity in two studied functional traits: SLA – specific leaf area, LDMC – leaf dry matter content. Explanation: The arrows represent the shift of centroid for the particular species between the control and experimental sites (the beginning of the arrow marks the centroid for control site and the end of the arrow marks the centroid for experimental site). PAR – photosynthetically active radiation, SOM – soil organic matter. For explanation of plant species and study sites, see Table 1

The first two axes of the MDS represent 85.6% of the total variability in trait values of analysed species. The changes in LDMC were more or less identical with the gradient of the first ordination axis, whereas the changes in SLA related to the gradient of the first as well as the second ordination axis (Fig. 2b). The area of the prediction ellipses reflecting the species plasticity in two analyzed traits ranged from 0.05 to 1.03 (*Galium odoratum* – 0.05, *Impatiens glandulifera* – 0.06, *Solidago gigantea* – 0.07, *Plantago lanceolata* – 0.08, *Glechoma hederacea* – 0.1, *Aegopodium podagraria* – 0.14, *Hedera helix* – 0.14, *Hypericum perforatum* – 0.17, *Fragaria vesca* – 0.2, *Prunella vulgaris* – 0.23, *Tussilago farfara* – 0.34, *Geum urbanum* – 0.38, and *Urtica dioica* – 1.03) (Fig. 2a). The values of PAR and soil properties are given in Tables 1 and 2, respectively. The changes in PAR values can be identified with the gradient of the first as well as second ordination axis (Fig. 2c). Among the soil properties, only soil organic matter showed a significant effect. The changes in soil organic matter content were reflected especially in the gradient of the second ordination axis. Based on the result of the multiple regression, PAR explains five times more information in the first two ordination axes than soil organic matter content (Table 3).

Table 1

Medicinal plant species and mean values of photosynthetically active radiation (PAR) at control and experimental sites

Study site	Plant species	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
BG	Tfar, Plan, Apod, Sgig, Hper, Fves, Pvul, Gurb, Igla, Udio	1927 (12:00 h CET)
	Hhel	1178 (10:00 h CET)
	Ghed	179 (10:00 h CET)
	Godo	27.4 (10:00 h CET)
R	Fves, Pvul, Tfar	44.1 (10:20 h CET)
	Igla	366 (11:20 h CET)
	Sgig	758 (9:40 h CET)
	Apod	2.7 (11:50 h CET)
PB	Hhel, Gurb, Ghed, Udio, Godo	7.2 (9:00–10:00 h CET) – shade
		493 (9:00–10:00 h CET) – sunflecks
ST	Plan, Hper	1631 (11:00 h CET)

Abbreviations:

Study sites: BG – the Garden of Medicinal plants, Faculty of Pharmacy, Comenius University in Bratislava, R – a meadow in Bratislava, district Rača, PB – a forest in Bratislava, district Podunajské Biskupice, ST – a site located close to the Faculty of Electrical engineering and Information Technology STU (anthropogenic site)

Plant species: Apod – *Aegopodium podagraria* L., Fves – *Fragaria vesca* L., Godo – *Galium odoratum* (L.) Scop., Gurb – *Geum urbanum* L., Ghed – *Glechoma hederacea* L., Hhel – *Hedera helix* L., Hper – *Hypericum perforatum* L., Igla – *Impatiens glandulifera* Royle, Plan – *Plantago lanceolata* L., Pvul – *Prunella vulgaris* L., Sgig – *Solidago gigantea* Aiton, Tfar – *Tussilago farfara* L. and Udio – *Urtica dioica* L.

CET – Central European Time

Table 2

Soil properties on control and experimental sites with the occurrence of medicinal plant species. Values listed are average values of soil pH, soil organic carbon (SOC) and soil organic matter (SOM), contents of clay (< 0.002 mm), silt (0.002–0.05 mm) and sand (0.05–2 mm) fractions. For explanation of plant species and study sites, see Table 1

Study site	Plant species	pH (CaCl ₂)	SOC (%)	SOM (%)	Soil texture			Class
					Sand (%)	Silt (%)	Clay (%)	
BG	Tfar, Plan, Apod, Sgig, Hper, Fves, Pvul, Gurb, Igla, Hhel, Ghed, Godo	7.3	1.9	3.2	61.6	29.6	8.8	sandy loam
	Udio	6.7	12.2	21.0	69.3	23.9	6.8	sandy loam
R	Fves, Pvul, Tfar	6.6	1.6	2.8	60.6	30.8	8.6	sandy loam
	Igla	7.1	3.1	5.4	66.1	24.7	9.2	sandy loam
	Sgig	7.0	1.4	2.5	65.6	26.6	7.7	sandy loam
	Apod	7.1	2.1	3.6	68.4	23.6	8.1	sandy loam
PB	Hhel, Godo, Gurb	7.1	4.4	7.6	55.3	34.7	10.0	sandy loam
	Ghed, Udio	7.2	6.2	10.7	37.2	52.1	10.7	silt loam
ST	Plan, Hper	7.2	1.0	1.7	60.6	24.3	15.1	sandy loam

Table 3

Results of the MDS and multiple regression between each environmental variable and the first two ordination axes

Model: MDS (trait_mat, distance="gower") Variation				
Total				8.7
MDS_Axis_1				6.2
MDS_Axis_2				1.2
Supplementary variables	MDS_axis1	MDS_axis2	Coefficient of determination	Permuted r coefficient
PAR	0.69	0.71	0.55	< 0.01
SOM	-0.31	-0.95	0.11	< 0.01

Discussion

Medicinal plants are an important part of the world's flora. They are located on every continent and are still collected and used in folk medicine. Unfortunately, there is little scientific work on medicinal plants. Even if some aspects of medicinal plants are processed, they are mostly about using medicinal plants in current pharmacy or about folk medicine (e.g. Petrovska 2012, Gurib-Fakim 2006, Jamshidi-Kia et al. 2018, Van Wyk and Wink 2018). Since data concerning the functional traits of medicinal plants are scarce, our results significantly contribute to this topic.

Variation in leaf functional traits, especially in SLA and LDMC, have guided many studies of functional ecology, which have addressed important ecological correlations (Adler 2014, Pérez-Ramoz et al. 2019). At the organ level, specific leaf area (SLA) is well known to positively associate with the plant's relative growth rate. Based on the above-mentioned findings, we first divided the studied species into the functional groups whereby SLA was considered.

High leaf SLA represents a resource-acquisitive plant strategy, while a high leaf dry matter content (LDMC) represents a resource-conservative strategy (Lambers and Poorter 1992). Species with low LDMC tend to be associated with productive, often highly disturbed environments. In cases where leaf area is difficult to measure (see above), LDMC may give more meaningful results than SLA, although the two traits may not capture exactly the same functions (Smart et al. 2017).

Results published by Majeková et al. (2014) confirmed the connection between plant functional traits and population temporal stability, whereby population temporal stability, measured as a coefficient of variation of species' biomass over time, was related to plant traits (including SLA and LDMC) covering different functional trade-offs. Plant functional traits linked to the leaf economic spectrum are important predictors of population stability regardless of both the abiotic and biotic conditions in which plants grew and species phylogenetic relatedness. High values of LDMC are associated with greater temporal

stability, indicating that slow-growing species with more conservative economics are generally more stable over time. Leaves with high LDMC tend to be relatively tough and are thus assumed to be more resistant to physical hazards (e.g., herbivores, wind, hail) than leaves with low LDMC (Cornelissen et al. 2003).

Wild medicinal plant species as a substantial component of natural plant communities contribute to the biodiversity and stability of natural ecosystems. Since medicinal plants are frequently exposed to various environmental stresses in their natural conditions, they have evolved physiological, biochemical, and molecular mechanisms to respond to harmful effects of these stresses (Masarovičová et al. 2019). The present results of SLA and LDMC (Fig. 1-3) characterized studied medicinal plants from the perspective of leaf functional traits that contribute to our understanding of their position in natural plant communities.

The next step in our research was development of plant functional groups using SLA and LDMC values (Fig. 1). Considering the importance of ecological effects on plants and vegetation, the summer period (June – August) 2018 and 2019 as well, in Slovakia could be characterized as relatively warm. From the meteorological perspective, sunny and warm weather was favourable for photosynthesis and growth of the studied medicinal plant species grown in both the botanical garden and natural communities. However, sunny and warm weather is usually accompanied by drought, especially under natural conditions, like in our experiments.

Plant communities differ in light, nutrients, and water availability, which are important factors in the selection and differentiation of which leaf traits should be used as indicators of change in the environment (Amaral et al. 2021). Based on the relationships between SLA and LDMC we focused on the two ecological factors: photosynthetically active radiation (PAR) and soil organic matter (SOM). SLA values increase with decreasing PAR and nutrient availability, which have been confirmed by the results of our research. There is a strong link between these two abiotic factors, while leaf blade thickness and mesophyll thickness increase with increasing PAR and nutrient availability without interaction. Photosynthetically active radiation is a vital source of energy for plants, and plants compete for this source, especially in dense communities. Plants have a variety of photosensory receptors through which they can detect the presence of competing species and subsequently adapt their growth and development strategies (Fiorucci and Fankhauser 2017). The availability of solar energy is a major ecological factor determining the convergence of leaf characteristics in the plant community (Gitelson et al. 2021), with no apparent effect of soil moisture on leaf characteristics (at the stand level), despite the importance of water in the drought-prone ecosystems (Ackerly 2003). The content of organic matter is an important parameter that indicates the overall quality of the soil, as well. It is influenced by many factors (vegetation, climatic conditions, soil type) (Scharlemann et al. 2014, Wan et al. 2019, Taghizadeh-Mehrjardi et al. 2020). In general, it depends mainly on the physical, chemical and microbiological properties (Rasmussen et al. 2018, Massaccesi et al. 2020) of the soil and has a positive effect on plant growth and biomass production (Lal 2020, Prommer et al. 2020, Anacker et al. 2021).

The ordination diagram (Fig. 1a) shows that in two functional groups with the lowest SLA values includes species growing on the control site (BG) under the highest PAR values ($1927 \mu\text{mol m}^{-2} \text{s}^{-1}$) except *Hedera helix* that occurred at $1178 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Ratjen and Kage (2013), for a single leaf, found that SLA is negatively correlated with light intensity as shown in our results.

Species with the medium values of SLA were assigned to the third and fourth functional group (Fig. 1a) and occurred at sites (BG), (R), (ST), and (PB). According to Lambers and Poorter (1992), these species would have intermediate potential relative growth rates.

The fifth and sixth functional groups presented species with the highest SLA values (Fig. 1a) that occurred mainly on site (PB). These results correspond with the findings of Cornelissen et al. (2003) that species growing in the herb layer of a forest are shade-tolerant with high values of SLA. These species also have a high potential relative growth rate. To these functional groups belong, in addition to species from site (PB), two species from site (BG) – *Galium odoratum* and *Glechoma hederacea*. These species were grown under a lower PAR level than the other species at the (BG) site. *Aegopodium podagraria* occurred on the (R) site with the lowest measured PAR value ($2.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ (11:50 h), which was manifested in relatively high SLA values (Fig. 1a). *Impatiens glandulifera* plants grown at site (R) with a mean value of PAR $366 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Species from site (BG) had, in general, lower values of SLA in comparison with plants from other sites. The highest values of this parameter were shown by *Glechoma hederacea* (PB), indicating the best adaptation of this species to the low irradiance level. This finding agrees with results in Lambers and Oliveira (2019) that plants growing in shade invest relatively more resources into LA, and these leaves are thin with high SLA values.

We divided the studied species into the functional groups according to the LDMC (Fig. 1b) parameters and their 95 % confidence intervals in accordance with bootstrapping values.

In the first functional group were assigned species with the lowest LDMC values (Fig. 1b). These species would possess the highest potential relative growth rate of all studied species. Leaf dry matter content is related to the average density of the leaf tissues (it is also related to leaf thickness) (Smart et al. 2017) and tends to scale with $1/\text{SLA}$. It has been shown that LDMC correlates negatively with potential relative growth rate (Lambers and Poorter 1992) and positively with leaf life-span (Cornelissen et al. 2003) and net primary production of aboveground biomass (Wang 2007), but the strengths of these relationships are usually weaker than those involving SLA (because $1/\text{SLA}$ combines leaf thickness and leaf density) (Lambers and Poorter 1992, Smart et al. 2017). This finding was not confirmed for the first functional group because to this group belong four species with summer leaves – *Plantago lanceolata*, *Tussilago farfara*, *Impatiens glandulifera*, and *Aegopodium podagraria* and only two species with evergreen leaves – *Glechoma hederacea*, and *Galium odoratum* (Klotz and Kühn 2002). Species with low LDMC tend to be associated with productive, often disturbed environments.

The second functional group comprised four species: *Galium odoratum* (BG), *Geum urbanum* (PB), and *Prunella vulgaris* (R) with evergreen leaves and *Urtica dioica* (PB) with summer leaves (Klotz and Kühn 2002). Results published by Majeková et al. (2014) documented a connection between plant functional traits and population temporal stability. Plant functional traits linked to the leaf economic spectrum are important predictors of population stability regardless of both abiotic and biotic conditions in which species grow and species phylogenetic relatedness. High values of LDMC are associated with greater temporal stability, indicating that slow-growing species with more conservative economics are generally more stable over time.

The third functional group included species with summer leaves (*Aegopodium podagraria*, *Hypericum perforatum*, *Solidago gigantea*, and *Urtica dioica*), and four species with evergreen leaves (*Prunella vulgaris*, *Hedera helix*, *Fragaria vesca*, and *Geum urbanum*) (Klotz and Kühn 2002). According to McIntyre (2008), a conservative strategy of plant species for low-productive undisturbed habitats relates to low SLA and high LDMC in contrast to fertile disturbed habitats, which select for high SLA and low LDMC. Leaf characteristics are useful in quantifying the links between vegetation change and ecosystem function that will be a vital part of ecosystem value assessments.

The last functional group included two species: *Hedera helix*, and *Fragaria vesca*, having the highest values of LDMC (Fig. 1b). Leaves with high LDMC tend to be relatively tough (Cornelissen et al. 2003, Smart et al. 2017) and are thus assumed to be more resistant to physical hazards (e.g., herbivores, wind, hail) than leaves with low LDMC. Additionally, leaves of *Hedera helix* are scleromorphic, and leaves of *Fragaria vesca* are mesomorphic (Klotz and Kühn 2002). Since LDMC is negatively correlated with relative growth rate (Lambers and Poorter 1992), we suppose that the above-mentioned species, compared with all studied species, has grown with the slowest rate. Since leaves of these species are evergreen, LDMC showed a positive correlation with the lifespan of the leaves (Klotz and Kühn 2002).

Since seasonal plasticity may enable plants to cope with adverse environmental conditions (Pérez-Ramoz et al. 2019, Stotz et al. 2021) and/or resource variability (Zunzunegui et al. 2011, Wang et al. 2019), we studied the plasticity of the plants using MDS (Fig. 2). More plastic species (e.g., *Urtica dioica*, and *Geum urbanum*) might show a greater ability for adaptation to ecological conditions. Similarly, *Galium odoratum*, *Impatiens glandulifera*, and *Solidago gigantea* are plastic species under given environmental conditions.

We performed a more complex differentiation of the examined species, when we used the metric multidimensional scaling (MDS) (Fig. 3). The first functional group includes *Plantago lanceolata* (BG), and *Tussilago farfara* (BG). According to Klotz and Kühn (2002), the species of this first functional group belong to C-S-R plant strategists. The values for individual taxa were modified and extended for the Czech flora by Chytrý et al. (2018, 2021).

The species of the second functional group were: *Impatiens glandulifera* (R), *Glechoma hederacea* (PB), *Glechoma hederacea* (BG), *Aegopodium podagraria* (R), *Galium odoratum* (PB), *G. odoratum* (BG), *Geum urbanum* (PB), and *Urtica dioica* (PB). However, these species have different strategies. *Geum urbanum*,

and *Glechoma hederacea* follow C-S-R strategies. *Impatiens glandulifera* is C-R, *Aegopodium podagraria*, and *Urtica dioica* are C-strategists, and *Galium odoratum* follows the S-strategy (Klotz and Kühn 2002).

Plantago lanceolata (ST), *Tussilago farfara* (R), and *Impatiens glandulifera* (BG) are included in the third functional group.

The fourth functional group includes: *Prunella vulgaris* (R), *Solidago gigantea* (BG), *S. gigantea* (R), *Hedera helix* (PB), *Hypericum perforatum* (BG), *Urtica dioica* (BG), and *Hypericum perforatum* (ST). *Prunella vulgaris* has a C-S-R strategy, *Hedera helix* follows a C-S strategy, whereas *Solidago gigantea*, *Hypericum perforatum*, and *Urtica dioica* exhibit a C-strategy (Klotz and Kühn 2002).

The fifth functional group includes only one species – *Fragaria vesca* (R). This species has a C-S-R strategy (Klotz and Kühn 2002).

Conclusions

Medicinal plant species comprise 21% of the Slovakian native flora. We chose these plant species because they are important component of both anthropogenic and natural plant communities not just in Slovakia, but all over the world as well. Species, we focused on, grew at a control site (botanical garden) and three natural sites (R, ST and PB) with different ecological conditions, where 13 wild medicinal species occurred. We described effects of irradiance and soil organic matter content on leaf functional traits, such as SLA and LDMC. The applied MDS method showed that PAR had a greater effect on the studied leaf traits than soil organic matter content. Moreover, we studied plasticity of these leaf functional traits and formed plant functional groups based on them. *Urtica dioica* and *Geum urbanum* possessed the greatest plasticity of all studied species.

Since plant species of a given functional group responded similarly to environmental variation, we expect them to respond similarly to ecosystem processes and habitat properties. Based on the functional groups we predict the response of the studied medicinal plant species to different ecological conditions. Traits and attributes of plants in plant communities are the outcome of adaptation of species to the environment and acclimation to environmental conditions changing in space and time. Above mentioned approach allowed us to understand and predict the behaviour of individuals and plant species populations in communities and functional interpretation and organisation of plant communities. We emphasize that the main mechanisms by which biodiversity affects stability of ecosystem functions all act through functional traits of organisms that form local communities. Therefore, identification of plant functional groups in a community can provide a better understanding and functional comparison of several communities than the classical approach based on taxonomy. Since data concerning the functional traits of medicinal plants are scarce, the present results will contribute to this field.

Declarations

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Figures

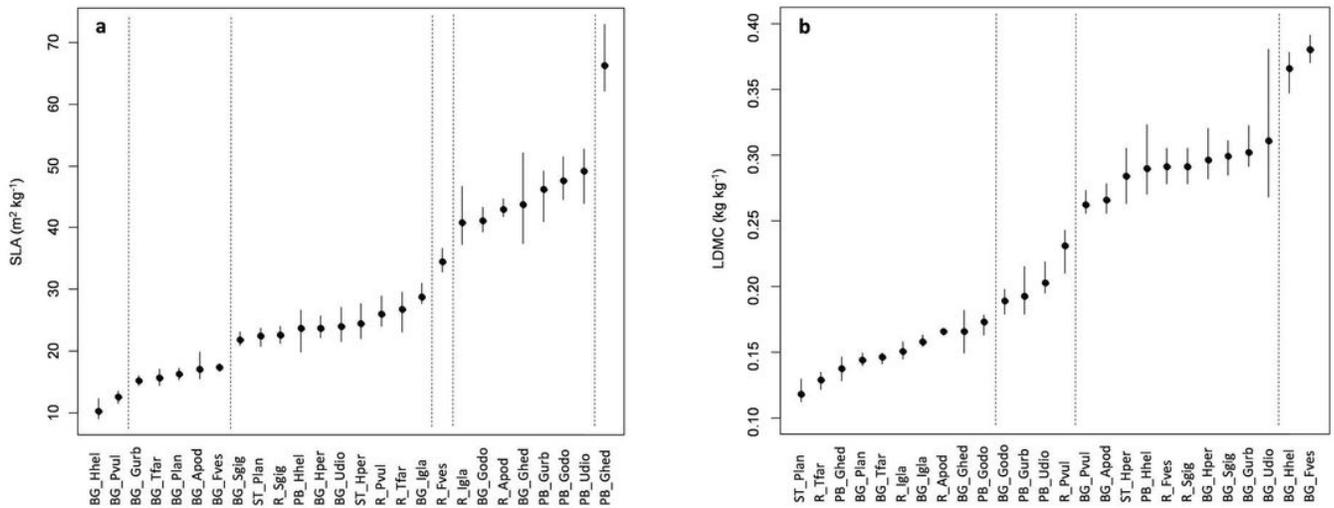


Figure 1

Mean values and their bootstrapped 95% confidence intervals of studied plant species on control and experimental sites. Species are ordered based on the increasing mean value of leaf traits at study sites. Dashed vertical lines identify groups of plants with overlapping 95% confidence intervals of the mean values of analysed leaf traits. SLA – specific leaf area and LDMC – leaf dry matter content. For explanation of plant species and study sites, see Table 1

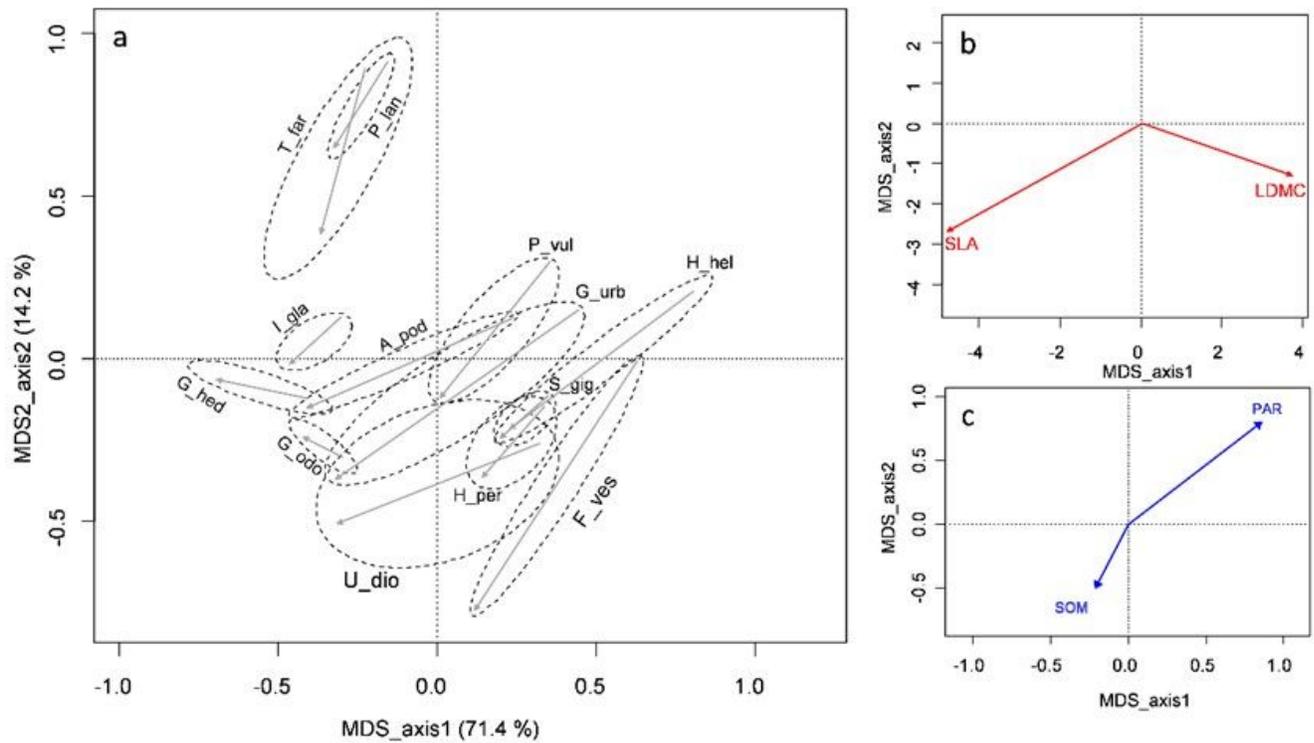


Figure 2

Results of the MDS showing the species plasticity in two studied functional traits: SLA – specific leaf area, LDMC – leaf dry matter content. Explanation: The arrows represent the shift of centroid for the particular species between the control and experimental sites (the beginning of the arrow marks the centroid for control site and the end of the arrow marks the centroid for experimental site). PAR – photosynthetically active radiation, SOM – soil organic matter. For explanation of plant species and study sites, see Table 1

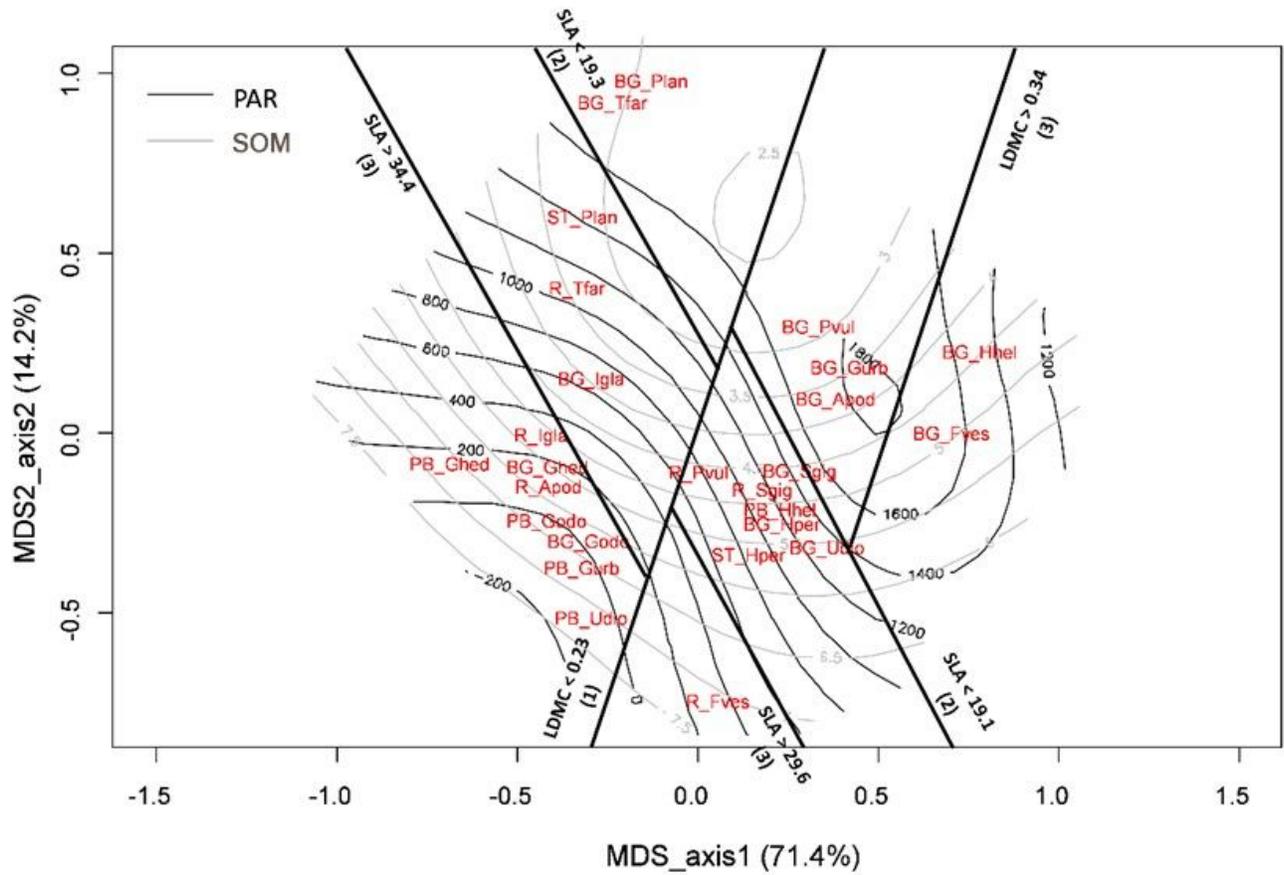


Figure 3

The plot of the MDS with the projection of the results of CART analysis. Position and classification of the studied plant species reflect values of two analysed leaf characteristics: SLA – specific leaf area ($\text{m}^2 \text{kg}^{-1}$) and LDMC – leaf dry matter content (kg kg^{-1}). PAR – photosynthetically active radiation, SOM – soil organic matter. For explanation of plant species and study sites, see Table 1